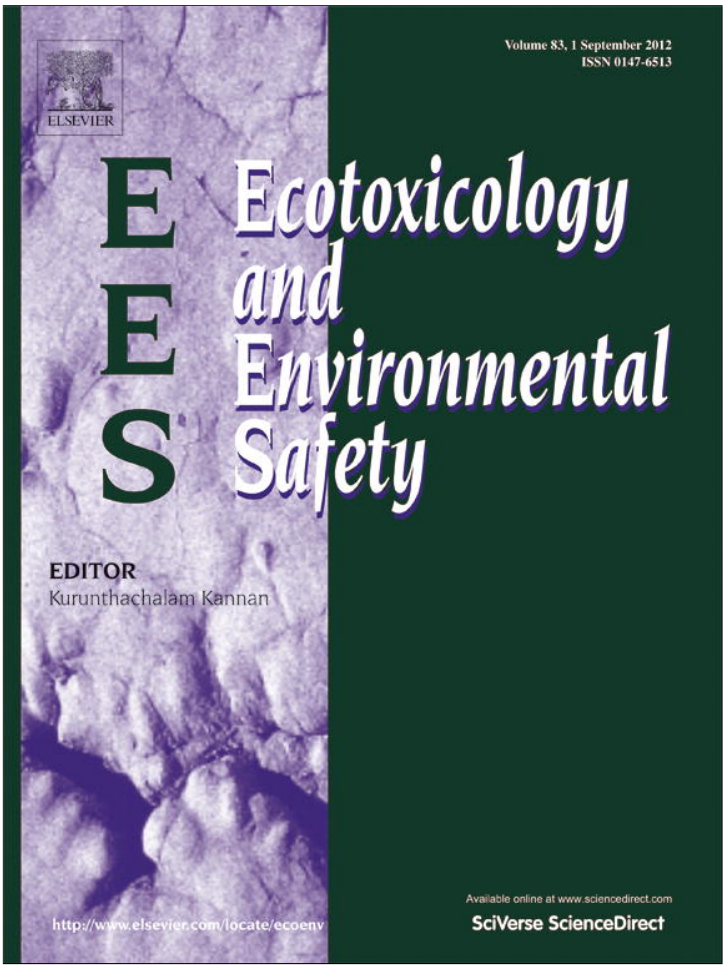

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Hexavalent chromium reduction, uptake and oxidative biomarkers in *Halimione portulacoides*

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ABSTRACT

The in situ reduction of Cr (VI) to its less toxic form Cr (III) may be a useful detoxification mechanism for phytoremediation. Using a hydroponics mesocosmos approach, we evaluated the ability of *Halimione portulacoides* to reduce and uptake Cr (VI) and its anti-oxidative feedback and biomarkers. It was found that this specie can, not only reduce large amounts of Cr (VI) in the external medium, but also withdrawn and accumulate this element in its roots and aboveground organs. Both these mechanisms were found to be dose dependent. Jointly with this phytoremediative potential the oxidative feedback was also assessed. Chromium uptake had its major implications on the chlorophyll content and flavonoid content, with potential consequences in the photosynthetic and photo-protective mechanisms. Although the high Cr root accumulation in *H. portulacoides*, there were no inactivation of the enzymatic defenses, allowing a continuous defense against reactive oxygen species. In fact, GPX and specially SOD revealed to be an excellent dose-related biomarker of Cr induced stress. All these aspects make this specie suitable for Cr (VI) phytoremediation processes, either by phytoextraction or by reduction of Cr (VI) to Cr (III) and also for monitoring programs using SOD and GPX as biomarkers of Cr environmental contamination.

1. Introduction

In the last decades phytoremediation as become a promising biotechnology for cleaning up contaminants, namely metals (Cunningham et al., 1995; Cunningham and Ow, 1996). Several works concerning a large variety of plant species have been published in the last decades identifying possible hyperaccumulator species (Salt et al., 1997; Brown et al., 1994). Besides accumulation some other abilities have arisen as potential metal detoxification mechanisms to less harmful forms, either by chelation (Duarte et al., 2007) or redox reactions, changing the metal's oxidation states. However two important aspects must be considered when choosing the best phytoremediator specie for a specific location and level of contamination: the biomass production ability and the ecology of the specie (Redondo-Gómez et al., 2011). When considering contaminated wetland phytoremediation, the number of potential species becomes reduced to a few due to the specificities of these environments, like tidal salt water flooding and waterlogging. One of the most abundant halophytic species of Tagus estuary salt marshes is *Halimione portulacoides*, previously described as a highly productive metal accumulator (Caçador et al., 2000; Duarte et al., 2007, 2009, 2010). Although it is considered a species with a phytoremediative potential, it is also important to consider its ecophysiological response (like oxidative feedback) to this metal accumulation. This oxidative feedback will be important to determine whether this specie can tolerate high Cr concentrations and with this maintain its ecophysiological health and carry out the phytoremediative process.

The nearby industries surrounding Tagus estuary had its activity peak from the 1970s to 1990s, with a widespread variety of industrial activities, from metallurgy to ship construction and others (Caçador et al., 2000). Chromium is used in many industrial processes and its unregulated use and dumping has led to water, sediment and biota contamination (Vale et al., 2008; Duarte et al., 2008, 2009). Industrial activities such as plating, tanning, corrosion inhibition, glassware-cleaning solutions, wood preservation, metal finishing or chromite ore processing (COP), where trivalent and hexavalent toxic chromium compounds are used (Barceloux, 1999; Losi et al., 1994). Cr elevated contents (up to 600 ppm) in some phosphate fertilizers may be also a significant source of this metal in soils, although the most hazardous addition of Cr to a soil is related to tannery sludges, which can contain up to 2.8% of this metal (Kabata-Pendias and Pendias, 2001). Estuarine areas are often affected by both these industrial and agricultural activities, unbalancing their critical environmental equilibrium (Pazos-Capeáns et al., 2010).

Chromium has two states of oxidation, Cr (III) and Cr (VI). The latter has been classified as a primary contaminant (Lytle et al., 1998) due to its mobility and reported harmful effects in animals and humans (Kortenkamp et al., 1996). Although this toxic effect associated to Cr (VI) form, Cr is also an essential element in the nutrition of several organisms in its stable Cr (III) form (Katz and

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Salem, 1994). This reduction of a toxic form to a stable nontoxic and beneficial form has gather the attention of several investigative teams. Abiotically the reduction of Cr (VI) can occur by reactions with other ions, metallic or mineral surfaces and organic molecules (Wittbrodt and Palmer, 1996). More important for phytoremediation proposes, the reduction of Cr (VI) to Cr (III) can be biologically mediated (Mikalsen et al., 1991; Stearns et al., 1995). Bacteria mediated Cr (VI) to Cr (III) reduction pathway by a specific Cr reductase is well-described (Shen and Wang, 1993; Nies, 1992; Romheld and Marschner, 1983), although this mechanism has not been identified in non-engineered plants.

The specific objectives of this study were to: (1) analyse the accumulation of Cr in plants subjected to experimental Cr (VI) treatments ranging from 0 to 30 mg^{-1} L; (2) assess the efficiency of this halophyte reducing Cr (VI) to Cr (III) (3) determine the oxidative feedback in order to understand if this specie tolerates high amounts of Cr while reducing it.

2. Material and methods

2.1. Plant collection and Treatments

H. portulacoides individuals were harvested in the northern margin of the Tagus estuary, near the Expo 98 exhibition site, at the end of the growing season. Samples were stored in plastic bags and quickly transported to the laboratory, where plants were washed with Milli-*Q* water to remove the dust and sediment. In order to allow new root development in the greenhouse, the roots and a small part of the stems were cut, leaving at least two nodes in the stem below the lowest branch. The individuals were placed in a greenhouse, at a temperature around 25 ± 5 °C, subjected to natural day/night regime, in dark-walled vases and filled with modified ¼ Hoagland nutrient solution, for approximately one and a half month to allow new root biomass growth. After the growth period, plants were washed and individualized in separate tubes. One group remained only with ¼ Hoagland solution; a second group was supplemented with 15 mg/L of potassium dichromate and finally a third group supplemented with 30 mg/L of potassium dichromate. These concentrations were chosen according to the available Cr recorded in Tagus salt marsh estuaries from previous studies (Duarte et al., 2008, 2009). Five individual replicates for each treatment were attained, as well as the same number of chromium solutions placed in the same conditions as control of spontaneous redox reactions. Samples from the different treatments were collected at day 0 and 7. Entire individuals were harvested at the end of the experiment and fractionated for analysis.

2.2. Biochemical analysis

For biochemical analysis only leafs from the middle nodes were harvested and immediately frozen in liquid N_2 and stored at −80 °C. Due to the biomass requirements for each biochemical analysis each leaf was only used for on type of analysis. All the harvested biomass was accounted. Due to the aerial and root biomass, the amount harvested for the biochemical analysis were found to be insignificant (less than 5% of the fresh biomass),

2.2.1. Estimation of lipid peroxidation

According to Heath and Packer (1968) the leaf samples were homogenized in 0.5% Tiobarbituric acid (TBA) containing 20% Trichloroacetic acid (TCA) at a ratio of 100:1 (m/v) leaf fresh weight to acid. The homogenate was extracted at 95 °C for 30 min and subsequently the reaction was immediately stopped in ice and centrifuged at $3000 \times g$ for 5 min at 4 °C. The absorbance of the supernatant was read at 532 nm and 600 nm in a Shimadzu UV-1603 spectrophotometer. The concentration of malondialdehyde (MDA) was calculated using the molar extinction coefficient, 155 mM^{-1} cm^{-1}.

2.2.2. Preparation of the crude enzyme extract

All enzymatic analyses were performed at 4 °C. Briefly, it was used a proportion of 500 mg of fresh plant material for 8 ml of 50 mM sodium phosphate buffer (pH 7.6) with 0.1 mM Na-Ethylenediamine tetraacetic acid (Na-EDTA), was employed for enzyme extraction. The homogenate was centrifuged at 14,000*g* for 20 min, at 4 °C, and the supernatant was used for the enzymatic assays.

2.2.3. Enzymatic assays

Catalase activity was measured according to the method of Teranishi et al. (1974), by monitoring the consumption of H_2O_2, and consequent decrease in absorbance at 240 nm. ($\varepsilon = 39.4$ mM^{-1} cm^{-1}). The reaction mixture contained 50 mM of sodium phosphate buffer (pH 7.6), 0.1 mM of Na-EDTA, and 100 mM of H_2O_2. The reaction was initiated with the addition of the extract. Ascorbate peroxidase was assayed according to Tiryakioglu et al. (2006). The reaction mixture contained 50 mM of sodium phosphate buffer (pH 7.0), 12 mM of H_2O_2, 0.25 mM L-ascorbate. The reaction was initiated with the addition of 100 μL of enzyme extract. The activity was recorded as the decrease in absorbance at 290 nm and the amount of ascorbate oxidized was calculated from the molar extinction coefficient of 2.8 mM^{-1} cm^{-1}. Guaiacol peroxidase was measured by the method of Bergmeyer et al. (1974) using a reaction mixture consisting of 50 mM of sodium phosphate buffer (pH 7.0), 2 mM of H_2O_2, and 20 mM of guaiacol. The reaction was initiated with the addition of 100 μL of enzyme extract. The enzymatic activity was measured by monitoring the increase in absorbance at 470 nm ($\varepsilon = 26.6$ mM^{-1} cm^{-1}). Superoxide dismutase activity was assayed according to Marklund and Marklund (1974) by monitoring the reduction of pyrogallol at 325 nm. The reaction mixture contained 50 mM of sodium phosphate buffer (pH 7.6), 0.1 mM of Na-EDTA, 3 mM of pyrogallol, Milli-Q water. The reaction was started with the addition of 100 μL of enzyme extract. Control assays were done in the absence of plant extract in order to evaluate the auto-oxidation of the substrates. All enzymatic activities were expressed as U^{-1} min^{-1} μg of protein.

2.2.4. Protein quantification

Proteins were determined according with Bradford (1976), using bovine serum albumin (BSA) as the standard protein.

2.2.5. Quantification of flavonoids and phenolics

For the extraction of phenolics and flavonoids, leaves were homogenized in methanol in the proportion of 1:100 (w/v) and centrifuged at 3000*g* for 10 min, at 4 °C. The quantification of the flavonoids was preformed according to the method described by Kumazawa et al. (2004). 1 ml of methanolic extract was added with 1 ml of ethanolic solution aluminium chloride 2% (w/v). The mixture was kept for 1 h in darkness, at room temperature, and then the readings were performed at 420 nm and compared with the calibration curve for quercitin. The flavonoid content was expressed as quercitin milli-equivalents per gram of fresh weight. Phenolics were quantified by the method of Folin and Ciocalteau (1927), using a reaction mixture consisting of 1 ml of methanolic extract, 5 ml of diluted Folin–Ciocalteu's phenol reagent (1:10 v/v) and 4 ml of sodium carbonate 7.5% (w/v). The mixture was also kept for 1 h in darkness at room temperature, and then the absorbance readings were performed at 750 nm and compared with the calibration curve for gallic acid (GA). Phenolic content was expressed as GA milli-equivalents per gram of fresh weight.

2.2.6. Total chlorophyll and carotenoids quantification

Photosynthetic pigments were extracted from freeze-dried leafs with 10 mL of pure methanol and kept overnight at 4 °C. The extract was centrifuge at $4000 \times g$ for 15 min at 4 °C, and chlorophyll and carotenoid (C_{x+c}) concentration were determined spectrophotometrically at 665.2, 652.4 and 470 nm, according to the method of Lichtenthaler and Wellburm (1983).

2.3. Chromium analysis

Chromium (VI) in the nutritive solution was analysed according to the Diphenylcarbazide (DPC) method. Briefly H_2SO_4 was added to 10 mL of solution until reaching a pH = 1. After acidification 1 mL of 0.2% DPC dissolved in acidic acetone (w/v) was added for red chromophore development. The concentration of Cr (VI) in solution was determined at 545 nm and compared with the calibration curve of made using potassium dichromate. Since the experiment was performed separately in a close system of replicate individuals, in the end of the experiment the amount of Cr III in solution could be determined as:

$$Cr(III) = Cr(VI)_i - (Cr(VI)_f + Cr_{plant})$$

where Cr $(VI)_i$ is the initial Cr (VI) concentration in the solution, Cr $(VI)_f$ is the final concentration in the solution at the end of the experiment and Cr_{plant} is the total chromium pool in the plant. To ensure that there were no Cr adsorption to the recipients walls, tube without plants were filled with Cr solutions during the same period. After the time trial the solution was discarded and the interior of the tube was washed with nitric acid and sent to analysis. The results showed there was no Cr in the acid washed from the tubes indicating that there was no adsorption to the tubes wall (data not shown).

After removal of the leaf samples used in the biochemical analysis, plants were separated into aerial parts and roots and completely dried at 60 °C until constant weight. All the plant material was reduced to powder with liquid nitrogen. Approximately 100 mg of each sample was digested for 3 h in a Teflon bomb, at 110 °C, by adding 2 ml of $HNO_3/HCLO_4$ (7:1, v/v). After cooling overnight, the extracts were filtered through Whatman No. 42 (2.5 μm of pore diameter) filters, diluted with Milli-*Q* water to a final volume of 10 ml and analysed by flame atomic absorption spectrometry (Perkin–Elmer A Analyst 100), using reference plant material, *Olea europaea* BCR62, according to the method described in Duarte et al. (2010).

2.4. Statistical analysis

Due to the lack of normality and homogeneity, the statistical analysis of the data was based in non-parametric tests. This way, the differences between treatments was preformed using Primer 6 software (Clarke and Gorley, 2006). Permanova coupled Monte Carlo tests were used to ensure a sufficient number of permutations (Clarke, 1993).

3. Results

3.1. Chromium uptake by H. portulacoides

Chromium uptake followed the increase of Cr supply in both root and aerial organs (Fig. 1). Root Cr content in plants supplied with both high and low doses of Cr showed a significant increase in this metal content when compared with the control individuals ($p<0.01$). Although the verified increase of 874 to 964 $\mu g\, g^{-1}$, respectively in the plants subjected to 15 and 30 $mg\, L^{-1}$, this difference was not significant ($p>0.1$). Concerning the aerial organs Cr accumulation statistically significant differences ($p<0.01$) were found between the control and each of the Cr treatments and between both Cr treatments.

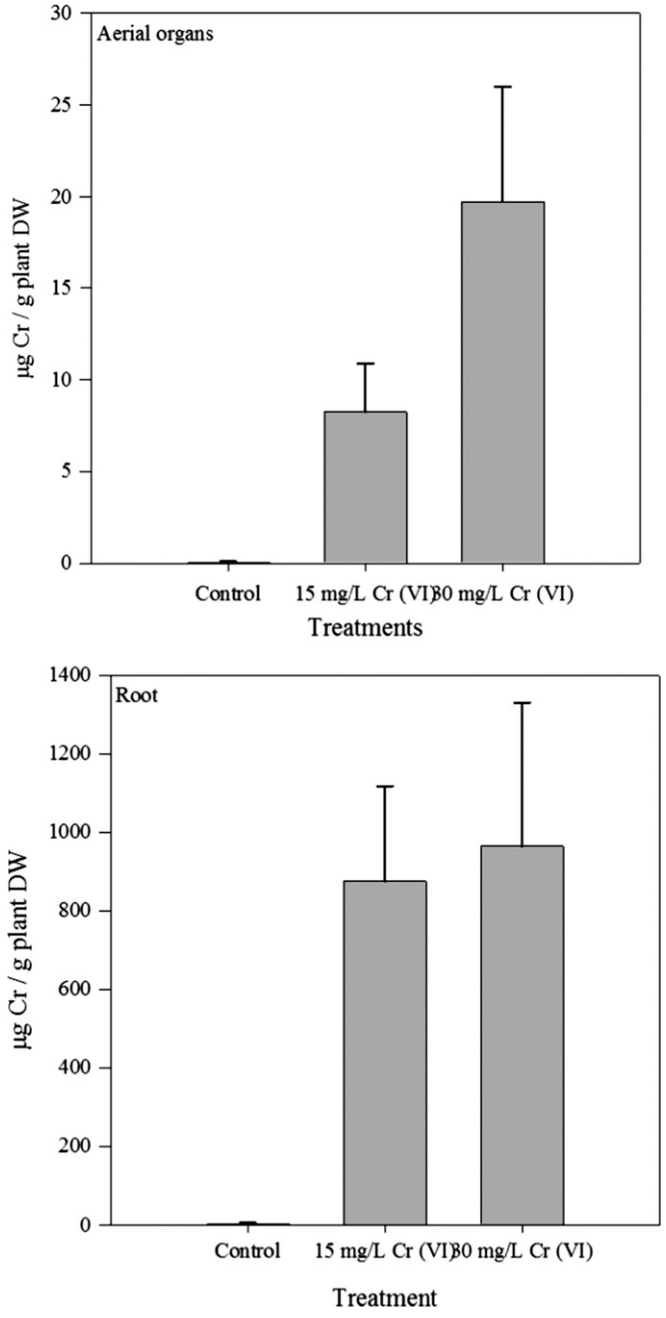

Fig. 1. Chromium concentration in *H. portulacoides* aerial organs and root tissues ($n=5$).

3.2. Chromium (VI) reduction

Considering the Cr pool in the plant tissues and also the Cr supply in the culture medium and its ionic form it was possible to assess the influence of *H. portulacoides* in the Cr (VI) to Cr (III) conversion, in the culture medium. As it is possible to verify by analysing Fig. 2, there are significant differences in the percentages of Cr (III) to Cr (VI) conversion between both treatments ($p<0.01$). In the medium supplied with 15 $mg\, L^{-1}$ of Cr (VI) about 40% of this element was converted to its less toxic form, while in the medium supplied with 30 $mg\, L^{-1}$ of the same toxic element about 60% was converted into Cr (III). Simultaneously to the plant Cr reduction and uptake assay, on two groups of five pots each containing the tested Cr (VI) concentrations were exposed to the same conditions that the plant pots, during the same period of time. In these pots there were not verified any Cr (VI) to Cr (III) auto-reduction reactions and the Cr (VI) concentration remained undisturbed, eliminating this way the possibility of spontaneous conversion reactions (data not shown).

3.3. Anti-oxidative biomarkers

Membrane damage, evaluated throughout the quantification of lipid peroxidation products (Fig. 3A), showed no significant differences among all the analysed individuals with and without Cr supply ($p>0.1$). Comparing the phenolic concentration in the different individuals leaf tissues, there were not also statistically significant differences among treatments, although the small decrease observed in Fig. 3B ($p>0.1$). As for the flavonolic content (Fig. 3C) there was a more evident trend in the content of these anti-oxidant compounds, decreasing with the increase of Cr (VI) supply. These differences were found to be statistically significant ($p<0.01$) when comparing both the individuals subjected to different Cr (VI) doses with the control situation but also among individuals treated with different Cr (VI) concentrations.

Concerning the anti-oxidant enzymatic mechanisms, their activity was evaluated in both leaf and root tissues. Root tissues

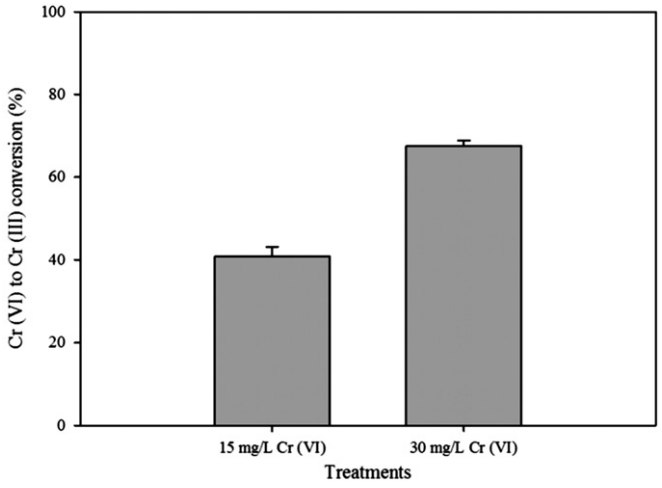

Fig. 2. Cr (VI) to Cr (III) conversion percentage in both the considered treatments ($n=5$).

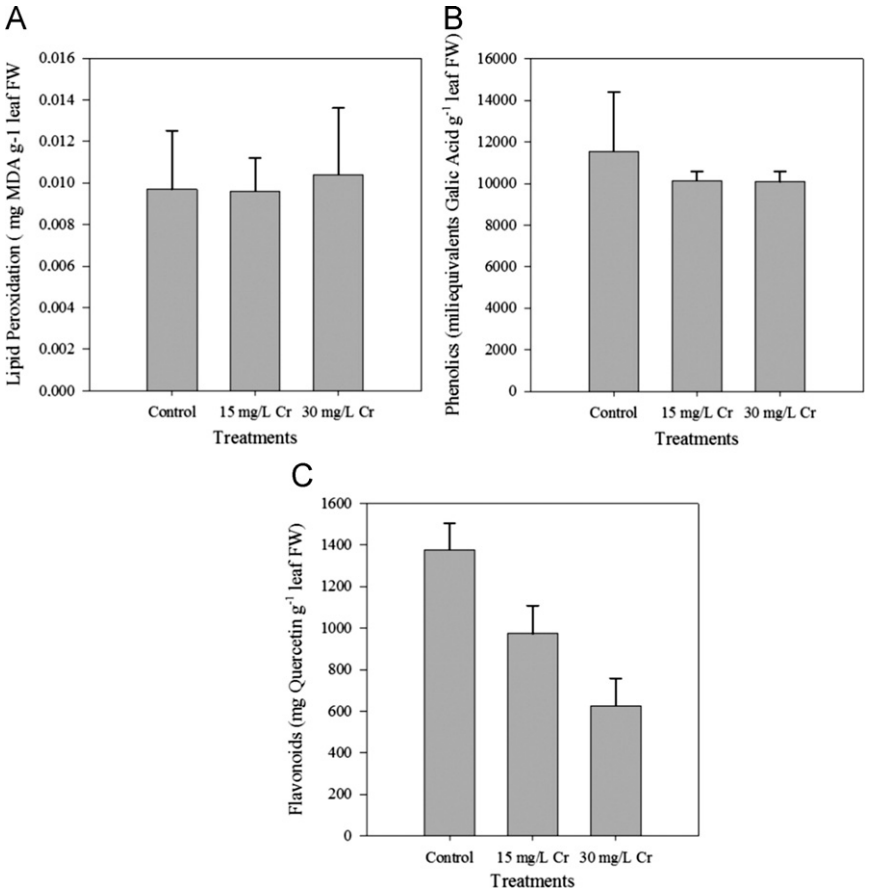

Fig. 3. Lipid peroxidation (A), phenolic (B) and flavonoid content (C) in *H. portulacoides* leaves subjected to different Cr (VI) treatments ($n=5$).

exhibited higher values of APX, GPX and SOD activities while CAT showed higher values in leafs for the control and Cr (VI) 15 mg L^{-1} treatments. CAT activity in leaf tissues (Fig. 4) showed an activity decrease with the increasing dose of Cr (VI), although it was not statistically significant. A similar pattern was verified for CAT activity in the root tissues (Fig. 4), with some differences in the individuals exposed to high Cr (VI) concentrations presenting an increase of CAT activity ($p > 0.01$). As for GPX activity in root tissues, it could be observed an increase of this parameter with the increasing Cr (VI) dose, although a small decrease could be assessed at the lower Cr (VI) concentrations. This difference of activity between the two tested Cr (VI) supplies was found to be significant ($p < 0.01$). Observing GPX activity in leaves it was found a slight increase of this enzyme activity in the individuals treated with the lower dose of Cr (VI), although there was a high variability between samples from the same treatments and no statistically differences between treatment groups. Regarding SOD activity, Cr (VI) supply led to a dose related activity increase in the root tissues, with very significant differences when comparing both with the control group and between Cr treatments ($p < 0.01$). In leaf tissues the inverse pattern was observed. The SOD activity showed a marked decrease ($p < 0.01$) in leaves of the individuals exposed to both concentrations of Cr (VI) in comparison with the control group.

3.4. Total chlorophyll and carotenoid leaf content

Analysing the total chlorophyll content in the leaves of Cr exposed individuals it was possible to observe a Chl reduction, when compared with the non-exposed individuals (control group). Although there were no statistically significant differences between the chlorophyll content among Cr (VI) treatments, when comparing either one of the Cr (VI) treatment groups with the control individuals the dissimilarities significantly increased ($p < 0.01$). Overlooking carotenoid content, the opposite trend was found. Although control individuals and plants exposed to lower doses Cr (VI) had very similar carotenoid contents ($p > 0.01$), when analysing the variation in the individuals exposed to higher metal concentrations, carotenoid increased significantly ($p < 0.01$) Fig. 5.

4. Discussion

The present study focused two main aspects of halophytes Cr (VI) exposure: its transformation and uptake and its ecophysiological consequences and biomarkers. This has implications both at ecological and cellular levels. Ecologically it was found that *H. portulacoides* could accumulate very high Cr concentrations, in particular in the root system. This is in agreement with earlier reports on rooted aquatic plants, which tend to accumulate Cr from the externally supplied metal solution (Gupta et al., 1999; Sinha et al., 2002; Suseela et al., 2002) and registered significantly higher values in the roots than their upper parts. High metal accumulation in the fine roots is also in agreement with earlier reports (Sinicrope et al., 1992). Qian et al. (1999) also reported highest concentration of Cr in the plant roots and lower level in shoots similarly to other ten elements studied in twelve aquatic plants. Also field-monitoring studies directed to this specie showed that the main biological metal sink is the halophytes root

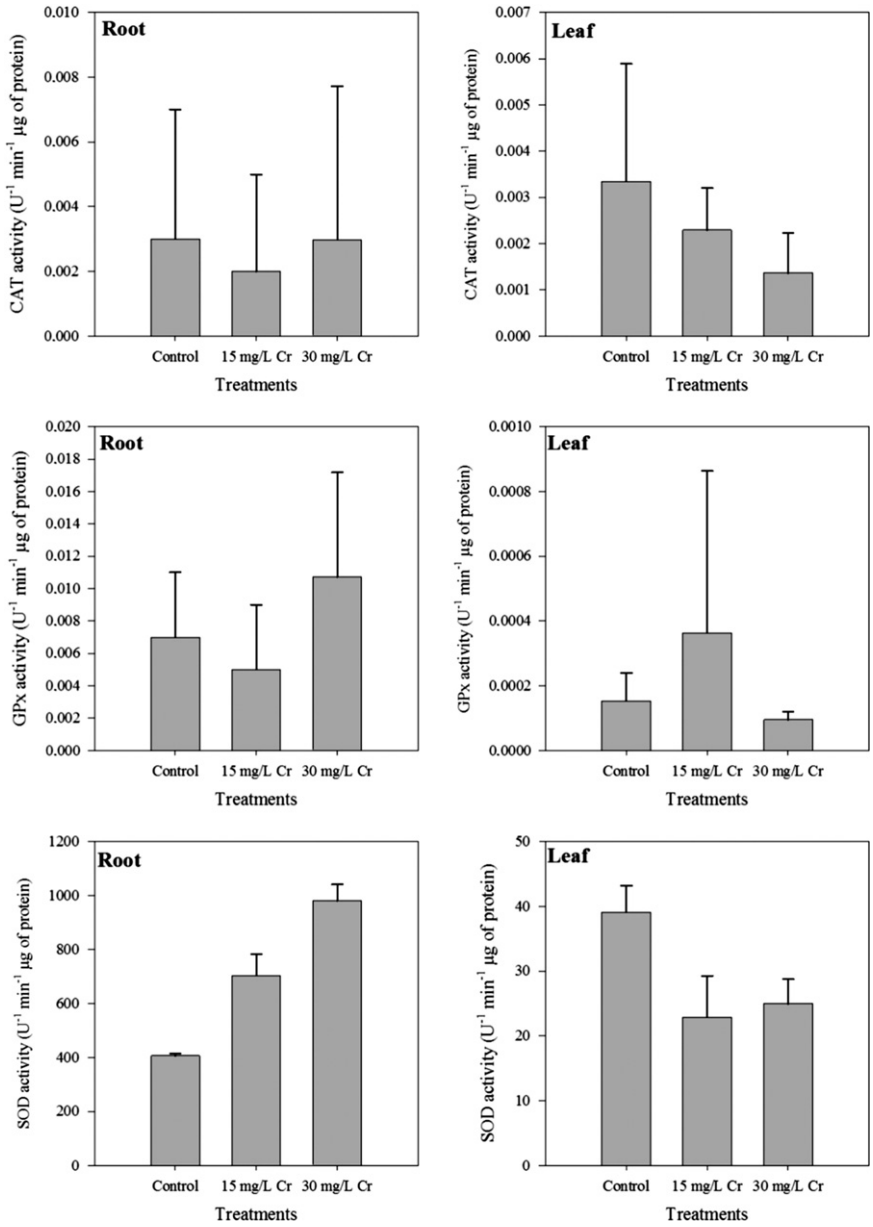

Fig. 4. Antioxidant enzymatic activities (CAT, GPX and SOD) in *H. portulacoides* root and leaves subjected to different Cr (VI) treatments ($n=5$).

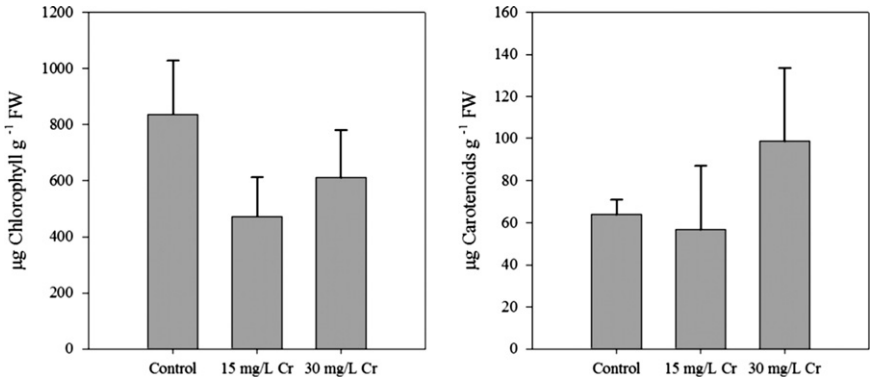

Fig. 5. Total chlorophyll and carotenoid content in *H. portulacoides* leaves subjected to different Cr (VI) treatments ($n=5$).

system (Caçador et al., 2009; Duarte et al., 2010). This can be due to metal binding to organic ligands and thus reducing its mobility from roots to aerial parts. Another important aspect focused in this study was the *H. portulacoides* mediated Cr (VI) to Cr (III) reduction. It was found that this specie can convert large Cr (VI) amounts to its less toxic form. This was already pointed out as defense mechanism

in sediments colonized by *H. portulacoides*, where a reduction of Cr (VI) to Cr (III) would have as consequence the retention, of this element, in Fe oxyhydroxide fraction, decreasing its bioavailability (Tanackovic et al., 2008). Lytle et al. (1998) found similar data concerning soluble Cr (VI) reduction by water hyacinth, suggesting that wetland plants uptake Cr in it less toxic form, throughout external reduction of Cr (VI) by their lateral fine roots, probably due to oxalate exudation. This points out for two potential phytoremediative applications. Not only this specie can accumulate large amounts of Cr withdrawn from its surrounding medium but it can also converts high percentages of the remaining Cr (VI) into a less toxic form. This toxicity reduction is important not only for this plant specie but also for the remaining surrounding biota, with a potentially essential role for environmental detoxification. This phytoremediation potential must be allied to a healthy and tolerant metabolism.

Metal accumulation at a cellular level is known to induce the reactive oxygen species (ROS) formation and may result in significant cellular damage. Membrane lipids are especially prone to attack by free radicals and are considered reliable plant oxidative stress indicators (Halliwell and Gutteridge, 1993; Palma et al., 2002). In this study there were no evident membrane damage in the photosynthetic organs. Although the areal organs Cr accumulation increase with the Cr supply, the main sink remained in the roots, alleviating Cr membrane damage in leaves tissues. Cells are normally protected against ROS by the intricate antioxidant systems operation, comprising both enzymatic systems such as superoxide dismutase (SOD), ascorbate peroxidase (APX), guiacol peroxidase (GPX) and non-enzymatic systems, such as free radical scavengers such as ascorbic acid, thiols, cysteine (Halliwell and Gutteridge, 1993), phenolics and flavonoids. The last two are known to be efficient anti-oxidant compounds in metal stress situations, but in case of Cr toxicity the information is rather scarce. Although phenolic content did not respond to Cr treatments, there is an evident pattern in flavonoid content decrease with Cr dose increase. Some recent studies regarding biosensors point out to quercetin-like flavonoids oxidation induced by Cr presence (Hosseini and Belador, 2009). In this case, flavonoids appear as biomarkers of Cr-induced stress reducing its content in Cr contaminated tissues, instead of being an antioxidant defense, as reported in similar studies.

As for ROS enzymatic defenses, SOD appears as a first line of defense, converting superoxide radicals to H_2O_2. Chromium mediated enhancement of SOD root activity found in this study may be due to either direct metal effect on SOD coding-genes or to an indirect effect mediated via superoxide radicals concentration increase (Chongpraditnum et al., 1992). Overlooking the SOD activity in leaves, the inverse pattern suggests a different SOD isoenzymatic form. In some cases it is reported that Cr can impair SOD activity by substituting the metallic ion in its reaction center (Stroinski and Kozlowska, 1997), concomitant with the activity reduction verified in the leaf tissues. Peroxidases are known to play a significance role in oxidative stress conditions and it has been shown that peroxidase activity can be used as a potential biomarker for sublethal metal toxicity (Radotic et al., 2000). Among H_2O_2 destroying enzymes, GPX activity was found to increase in roots along with the Cr supply. Shah et al. (2001) also reported similar findings showing increase in GPX activity in metal treated plants. The GPX activity increase could be a consequence of either the microenvironment or the tissue specific gene expression in the treated plants (Hegedus et al., 2001). Another important H_2O_2 detoxification mechanism is CAT activity. While in roots there were no differences in CAT activity among treatments, in leaves CAT activity decreased with the supplied Cr dose. This was already reported in similar studies with other plant species (Shanker et al., 2005). The CAT activity decrease ue to accumulated chromium has a inhibitory effect on the protein synthesis pathway.

Overlooking total chlorophyll concentration, a decrease in this pigment concentration could be observed. Chlorophyll biosynthesis inhibition is well reported in metal stressed plants (Sinha et al., 2002, 2003), and is normally attributed to reduced *d*-aminolevulinic acid dehydratase (ALAD) activity (Padmaja et al., 1990). Besides chlorophyll biosynthesis inhibition Dhir et al. (2009) also pointed out the reduction in Fe content and/or replacement of central Mg^{2+} ion in the chlorophyll molecule as a possible cause for chlorophyll depletion. While chlorophylls are normally classified as light-harvesting pigments, carotenoids role is focused mainly on chlorophyll and photosystem photo-protection under stress conditions. These molecules are known to quench the photodynamics reactions that lead to chlorophylls loss, replace peroxidation and chloroplast membrane collapse (Knox and Dodge, 1985). In the present study, an increase in the carotenoid content was recorded with Cr dose increase, similarly to the observed in Cr treated *Vallisneria spiralis* individuals (Vajpayee et al., 2001), indicating a possible carotenoid quenching role in this case.

5. Conclusions

From this study two major conclusions arise from two distinct but correlated points of view: the ecological and the cellular one. From an ecological point of view, *H. portulacoides* revealed an ability to detoxify the surrounding medium, by transforming high percentages of Cr (VI) into Cr (III). This has implications in the estuarine system remediation, since this specie is not only one of the more abundant in the Portuguese salt marshes, but also for the food web and neighbour biota species. By itself, Cr uptake is also a process that affects *H. portulacoides* physiology, since it not only transforms the more toxic Cr form into a less harmful one, but this specie also withdraws large metal amounts form the system. Ecophysiologically this has as major implication, a chlorophyll content reduction that inevitably can affect this specie primary production. Also flavonoid content was affected, leading to a decrease in its content that can interfere with the photoprotection mechanisms provided by the anthocyanins and other flavonoid-derived molecules. Although the high root Cr storage, there was no enzymatic defences inactivation, by the contrary, GPX and especially SOD revealed to be an excellent dose-related biomarker of Cr induced stress. The positive feedback from the anti-oxidative enzymatic mechanisms simultaneously with high Cr (VI) to Cr (III) root accumulation and conversion, as primary storage and reaction tissue, make this specie suitable for Cr-contaminated sites phytoremediation, both by phyto-extraction and by rhizo-transformation of this metal more toxic forms.

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